9

OBSERVATIONS ON THE FACTORS CONTROLLING THE REPRODUCTION OF TWO COMMON SPECIES OF BROWN ALGAE, *COLPOMENIA PEREGRINA* AND *SCYTOSIPHON* SP. (SCYTOSIPHONACEAE), IN VICTORIA

By Margaret N. Clayton

Botany Department, Monash University, Clayton 3148

Abstract: Observations of seasonal changes in the mode of reproduction of *Colpomenia peregrina* (Sauvageau) Hamel and the complanate form of *Scytosiphon* in Victoria have been collected over several years. They are consistent with the results of experiments testing the effects of temperature and daylength on the reproduction of cultured plants which show that these environmental factors determine whether thalli give rise to similar (gametophytic) progeny or to the alternative sporophytic phases of the heteromorphic life histories. Such changes in the mode of reproduction form the basis of the seasonality of the larger gametophytes.

Sexual reproduction is a rare winter occurrence in both species, but experiments indicate that it is not simply controlled by temperature/daylength conditions. In culture, in conditions which favour the production of sporophyte progeny, *Scytosiphon* gametophytes began to release zooids at an age of 8-10 weeks but they showed no signs of sexuality; later, after 14 weeks, functional gametes were produced. The association of sexual maturity with age, a phenomenon previously unknown in this group of algae, helps to explain the elusive nature of sex in wild populations and its occurrence several months after the onset of the main growing season in autumn.

INTRODUCTION

Colpomenia peregrina (Sauvageau) Hamel and *Scytosiphon* sp. are brown algae with complex heteromorphic life histories. The well known saccate and complanate thalli are the gametophytic generations and under certain conditions they produce the small and inconspicuous crustose sporophytic generations. Gametophytes of both species are common in the intertidal region of Victorian coasts from May to November, and fertile plants can be found throughout this period. The gametophytes show seasonal changes in their mode of reproduction. During May and June they produce zooids which develop into more gametophytes, but from mid-June to September the majority of zooids give rise to crustose sporophytes. During September *C. peregrina* reverts to gametophyte production (Clayton 1979). A small proportion of the *Scytosiphon* sp. population also produces gametophytic progeny at this time of year, but the majority produce sporophytes. The *Scytosiphon* sp. gametophytes die out altogether in December (Clayton 1976b, 1980). The results of several years of observations on the reproduction of the two species (Figs 1 & 2) illustrate these seasonal changes.

(Note: *Scytosiphon* sp. refers to the taxon *S. lomentaria* var. *complanata* Rosenvinge. In Australia it has a life history which also includes a cylindrical form resembling *Scytosiphon lomentaria* (Lyngbye) Link (Clayton 1976a, b). Its taxonomic status is discussed in Clayton 1980.)

Experiments on the effects of daylength and temperature on the reproduction of cultured *Scytosiphon* sp. gametophytes indicated a possible basis for the seasonal changes outlined above. In daylengths of 8 hours some strains consistently reproduce gametophytic generations. Daylengths of 10 hours and longer induce sporophyte production, although gametophytic progeny were occasionally produced in daylengths of 12 hours. These responses of cultured *Scytosiphon* sp. to daylength are in broad agreement with the changing pattern of reproduction of wild *Scytosiphon* sp. and the corresponding seasonal changes in daylength (Figs 2 & 3). It may therefore be inferred that daylength is an important factor controlling reproduction in this species (Clayton

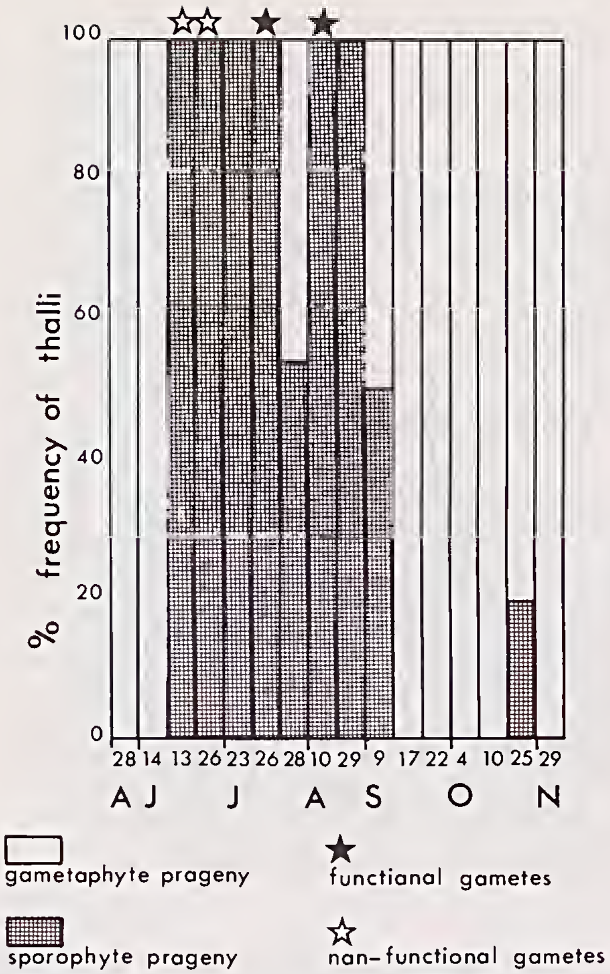

FIG. 1.—*Colpomenia peregrina*—seasonal changes in the percentage frequency of wild thalli producing gametophytic and sporophytic (or predominantly sporophytic) progeny.

1976b, 1980). A comparable influence could not be attributed to temperature.

A parallel series of observations on the effects of temperature and daylength on the reproduction of *C. peregrina* were carried out in order to compare the control of reproduction in a species having a different seasonal cycle from *Scytosiphon* sp. The results are described in this paper.

Sexual reproduction, which involves mating between anisogametes, is a rare winter occurrence in both species. Gametes are formed by some of those gametophytes which give rise to sporophytic progeny (Figs 1 & 2). Amongst cultured plants mating has only been observed a few times. *Scytosiphon* sp. produced gametes in cultures grown at 12°C, L:D 10:$\overline{14}$ and 12:$\overline{12}$h, and 14°C, L:D 10:$\overline{14}$h, but only a relatively small proportion of the zooids were functional gametes (Clayton 1980). These results indicate a possible relationship between 'winter' conditions and gamete formation in *Scytosiphon* sp., but both the rarity and the variable success of mating reactions suggest that other factors are involved. A series of observations was designed to investigate another factor which might affect gametogenesis namely the age of the parent individual. *Scytosiphon* sp., rather than *C. peregrina*, was selected as a subject for this study as a greater number of cultured strains of known sex were available.

METHODS OF INVESTIGATION

CULTURE METHODS

All cultures were grown in Provasoli's ES medium (Wynne 1969) prepared using millepore-filtered seawater, which was changed at intervals of approximately two weeks. Except where specifically mentioned, culture dishes (Pyrex No. 3250) containing about 150 ml medium were used. Overhead lighting was supplied by Sylvania Grolux wide spectrum fluorescent tubes giving a quantum irradiance of 60-70 μ E cm^{-2} s^{-1}.

THE EFFECTS OF TEMPERATURE AND DAYLENGTH ON THE REPRODUCTION OF *C. PEREGRINA*

The range of conditions investigated (Table 1) was chosen to include the temperatures and daylengths prevailing during the main growing season of *C. peregrina*, from autumn to early summer (Fig. 3). Initially four different strains

TABLE I

COLPOMENIA PEREGRINA—THE PERCENTAGE OF SPOROPHYTES IN THE PROGENY OF FOUR STRAINS CULTURED IN VARIOUS TEMPERATURE/DAYLENGTH REGIMES

	Strains			
Temperature/Daylength	1	2	3	4
12°C L:D 10:$\overline{14}$h	100	89	0	43
12°C L:D 12:$\overline{12}$h	>99	81	100	100
14°C L:D 10:$\overline{14}$h	>99	97	0	>99
14°C L:D 12:$\overline{12}$h	93	100	0	0
16°C L:D 8:$\overline{16}$h	93	100	0	0
16°C L:D 10:$\overline{14}$h	90	>99	0	0
16°C L:D 12:$\overline{12}$h	90	>99	died	0
16°C L:D 14:$\overline{10}$h	100	92	0	0

Original isolates
1. Flinders 26.7.77
2. Flinders 26.7.77
3. Flinders 17.9.77
4. San Remo 29.11.75

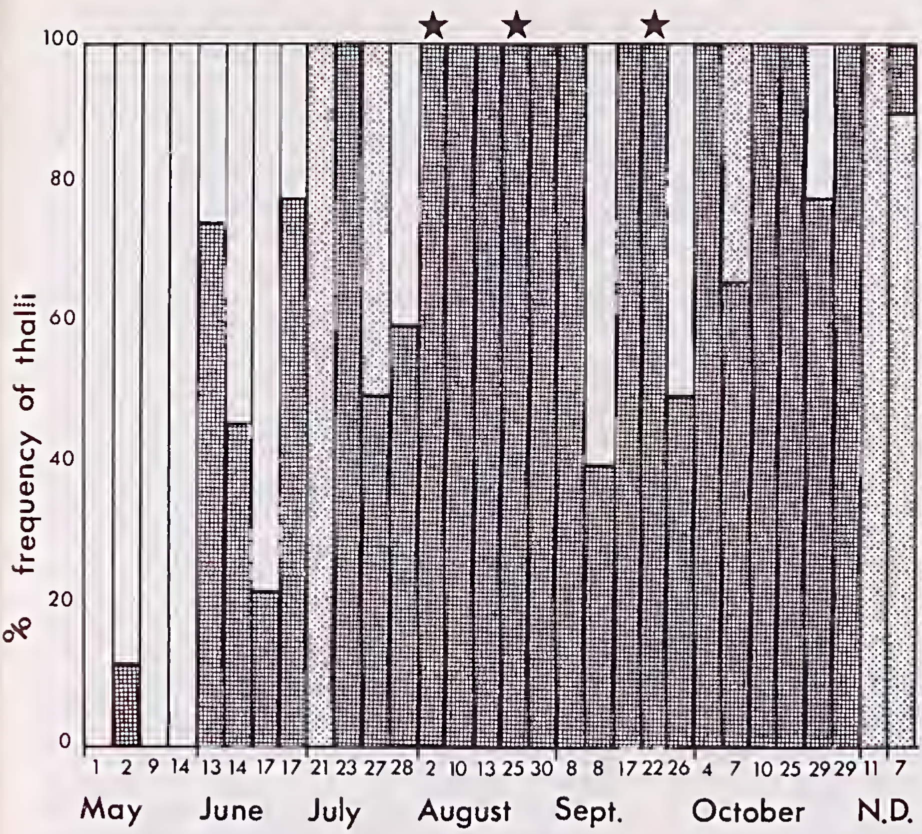

FIG. 2—*Scytosiphon* sp.—seasonal changes in the percentage frequency of wild complanate thalli producing gametophytic and sporophytic (or predominantly sporophytic) progeny. (Occasional cylindrical progeny are indicated by stippled shading. Their occurrence has been discussed in previous publications).

were tested. Cultures were inoculated with three very young gametophytic germlings, 7-14 days old. When sori were observed on the fronds, usually from an age of 6-8 weeks onwards, the culture dish was placed in a black plastic sleeve that masked half the contents. This enabled the phototactic response of the zooids to be determined, after which the sleeve and the parent thalli were removed. The progeny were cultured until it was clear whether they were gametophytes or sporophytes. When both were present the proportions of each were estimated by counting sample fields, or if possible the entire population using a stereo dissecting microscope.

GAMETOGENESIS IN CULTURED *SCYTOSIPHON* SP.

Thirteen strains were tested. The culture dishes were inoculated with single gametophytic germlings. They were grown at 14°C, L:D 10:14h, conditions approximating those in which wild *Scytosiphon* sp. produces gametes (Figs 2 & 3). From the time the plants became fertile their zooids were checked at weekly intervals and were matched with zooids from plants of the opposite sex. The presence or absence of mating reactions followed by the formation of zygotes was recorded (Table 2).

RESULTS

THE EFFECTS OF TEMPERATURE AND DAYLENGTH ON *C. PEREGRINA*

C. peregrina did not reproduce in the same way in all temperature/daylength conditions. Two strains produced a large proportion of sporophytic progeny in all conditions, and the other two did so only in three of the eight sets of conditions (Table 1), indicating that both daylength and temperature affect reproduction.

Subsequently, in conjunction with other studies of the effects of temperature and daylength on life history, five more strains of *C. peregrina* were cultured in four new temperature/daylength regimes (Table 3). The strains were of the kind which produced gametophytic progeny in most conditions. In all the test conditions they continued to produce gametophytes. Although different from the strains used in the first experiment, the results permit further definition of the environmental conditions which limit sporophyte production and the potential for sexual reproduction in *C. peregrina*.

The phototactic responses of *C. peregrina* zooids were in most instances not clear as large numbers of germlings developed in both darkened and light parts of the culture dishes. Under the

TABLE 2
SCYTOSIPHON sp. – THE AGE IN DAYS OF CULTURED GAMETOPHYTES WHEN THEY PRODUCED NON-SEXUAL ZOOIDS AND GAMETES

Cultured strains	Sex	Zooid production			Gamete production	
Flinders 2.8.76	i. F	44	61			
	ii. M	44	61		88	91
Flinders 15.8.78	i. F	78	101		128	132
	ii. M				115	132
	iii. F				132	
Flinders 19.9.78	i. F	60	67	84	98	
	ii.	67				
	iii. F	84			98	
	iv.	84				
	v. F	84			98	
	vi.	60	84			
	vii. F				98	
	viii. M				8	

same conditions *Scytosiphon* sp. zooids show a more definite phototaxy: negatively phototactic zooids develop into sporophytes and positively phototactic zooids develop into gametophytes (Clayton 1980).

GAMETOGENESIS IN CULTURED *SCYTOSIPHON* SP.

Four gametophytes produced only non-sexual zooids. Five plants initially released zooids which failed to mate with zooids from plants of the opposite sex, but on later occasions they released functional gametes (Table 2). All the zooids released by the four remaining strains included some gametes. The exact percentages of gametes which were successful in forming zygotes were not calculated but it was clearly only a fairly small proportion of those released.

DISCUSSION

The reproductive cycle of *C. peregrina*, like that of *Scytosiphon* sp., is controlled by environmental factors. It is significant that the conditions (12°C, L:D 10:14 and 12:12h, and 14°C L:D 10:14h) which induced sporophyte production in otherwise gametophyte-producing strains of *C. peregrina* correspond to the temperatures and daylengths prevalent in winter in Victoria (Fig. 3), the time of year when wild *C. peregrina* invariably produces mostly sporophytic progeny. The range of daylength in which gametophytes give rise to sporophytes is narrower than it is in *Scytosiphon* sp. (Figs 1 & 2) in which long days also induce sporophyte production. The ephemeral wild plants show a related difference in their seasonality: *C. peregrina*, although inconspicuous in summer, occurs throughout the year, whereas *Scytosiphon* sp. is absent from the shore between December and April.

The existence of strains of *C. peregrina* and *Scytosiphon* sp. that persistently produce sporophytes irrespective of temperature and daylength and others in which reproduction is affected by environmental conditions, has been discussed previously (Clayton 1979, 1980). The basis for this difference is at present obscure, but it should become apparent from detailed analyses of inheritance and life histories.

Previous research has shown that temperature and daylength may affect the life histories of a number of species in the Scytosiphonaceae in several ways (Wynne & Loiseaux 1976). An association between long days and sporophyte production has been demonstrated in the cylindrical *Scytosiphon lomentaria* (Lyngbye) Link (Wynne 1969; Nakamura & Tatewaki 1975). However, no study has been sufficiently detailed to provide a comprehensive definition of the range of conditions which determine the production of sporophytic and gametophytic progeny in any species.

In several species of the Scytosiphonaceae, gametes have been shown to develop parthenogenetically into sporophytes; a small number of parthenogametophytes occasionally also develop (Nakamura & Tatewaki 1975; Clayton 1979, 1980). In the brown algal species *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Müller 1967), and in the green alga *Ulva mutabilis* Føyn, (Fjeld & Løvlie 1976), parthenogenesis likewise leads to sporophyte development. The reasons are doubtless complex why many of the zooids from wild and cultured *Scytosiphon* sp. and *C. peregrina* which ultimately develop into sporophytes do not function as gametes, but one factor implicated by the present study of *Scytosiphon* sp. relates to the age of the gametophytes, a phenomenon which has not previously been reported. Unfortunately it is impossible to say whether the impotence of the first-formed zooids of some gametophytes was a character of one or both sexes, as functional male and female gametes were not available simultaneously for the appropriate checks to be made. It is interesting to note that the age of cultured *Scytosiphon* sp. on reaching sexual maturity corresponds approximately to the length of time wild gametophytes are present on the shore before they begin to release gametes in late winter. However, there is no information available on the rate of growth and the longevity of wild plants so this correlation must remain speculative. The subject is currently under investigation.

Even if age is a factor in the development of sexual competence in *Scytosiphon* sp., the fact remains that gametophytes are common on the shore for seven months of the year, they produce sporophytic progeny for the greater part of five months, and yet gamete-producing plants have been recorded during a period of no more than seven weeks in the winter and early spring. The same is also true of *C. peregrina* except that the period of sporophyte production is three months.

TABLE 3

EXPERIMENTAL CONDITIONS USED IN THE SECOND INVESTIGATION OF THE EFFECTS OF TEMPERATURE AND DAYLENGTH ON THE REPRODUCTION OF *COLPOMENIA PEREGRINA*

Temperature/Daylength

12°C L:D 10.5:$\overline{13.5}$h
14°C L:D 9.5:$\overline{14.5}$h
14°C L:D 12.5:$\overline{11.5}$h
16°C L:D 10.5:$\overline{13.5}$h

Original isolates

1.	Point Nepean	14. 6.78
2.	Sorrento	27. 7.78
3.	Flinders	22. 9.78
4.	Sorrento	10.10.78
5.	Flinders	25.10.78

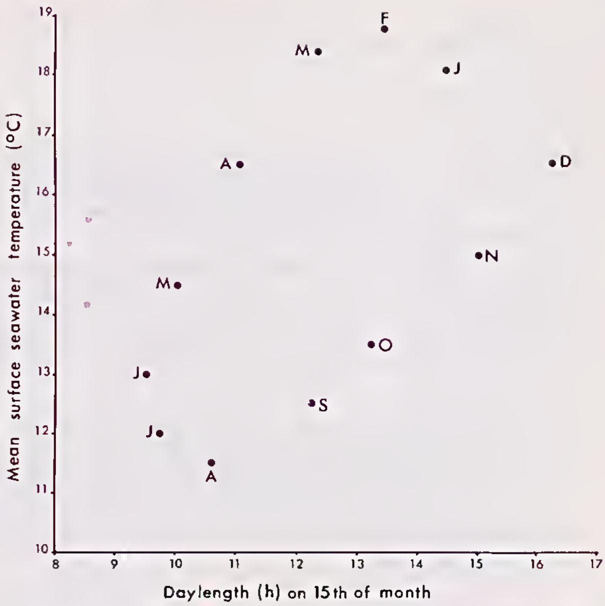

FIG. 3—Daylength and surface seawater temperatures throughout the year in Victoria. (Temperature data obtained from King 1970).

An age-maturity factor alone could not account for the transitory nature of sexuality. The interesting coincidence between the culture conditions in which *Scytosiphon sp.* produced functional gametes (Clayton 1980) and those which induced sporophyte production in *C. peregrina* suggests the possibility that gametogenesis in wild populations may be in some way precisely regulated by changes in environmental parameters such as temperature and daylength.

Many experimental studies of the Scytosiphonaceae have underlined the lack of dependence of the species upon sexual processes as a means of reproduction. For a large part of their growing season *Scytosiphon* sp. and *C. peregrina* reproduce asexually, and even when gametes are produced, many of them fail to form zygotes, but develop parthenogenetically. Furthermore, plants which are able to produce gametes may at some other stage in their development produce nonsexual zooids. It is possible that further studies may reveal selective factors which counterbalance this tendency towards asexual reproduction, as in, for example, *Ulva mutabilis* in which the zygotes have a significantly greater chance of survival than either zoospores or gametes (Løvlie & Bryhni 1978).

ACKNOWLEDGEMENTS

I wish to thank Mrs Iona Christianson and Mrs Mary Cole for technical assistance. The research was supported by a grant from the Australian Research Grants Committee.

REFERENCES

CLAYTON, M. N., 1976a. The morphology, anatomy and life history of a complanate form of *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyta) from southern Australia. *Mar. Biol.* 38: 201-208.

———, 1976b. Complanate *Scytosiphon lomentaria* (Lyngbye) J. Agardh (Scytosiphonales, Phaeophyta) from southern Australia: the effects of season, temperature and daylength on the life history. *J. exp. mar. Biol. Ecol.* 25: 187-198.

———, 1979. The life history and sexual reproduction of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyta) in Australia. *Br. phycol. J.* 14: 1-10.

———, 1980. Sexual reproduction—a rare occurrence in the life history of the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta) from southern Australia. *Br. phycol. J.* 15: 105-118.

FJELD, A. & LøVLIE, A., 1976. Genetics of multicellular marine algae. In *The Genetics of Algae*, pp. 219-235. R. A. Lewin (Ed.) Blackwell, Oxford.

KING, R. J., 1970. Surface sea-water temperatures at Port Phillip Bay Heads, Victoria. *Aust. J. mar. Freshwat. Res.* 21:47-50.

LøVLIE, A. & BRYNHI, E., 1978. On the relation between sexual and parthenogenetic reproduction in haplo-diplontic algae. *Bot. mar.* 21: 155-163.

MULLER, D. G., 1967. Generationswechsel, Kernphasenwechsel un Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta* 75: 39-54.

NAKAMURA, Y. & TATEWAKI, M., 1975. The life history of some species of the Scytosiphonales. *Scient. Rep. Inst. alg. Res. Fac. Sci. Hokkaido Univ.* 6: 57-93.

WYNNE, M. J., 1969. Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *Univ. Calif. Publs Bot.* 50: 1-62.

———, & LOISEAUX, S., 1976. Recent advances in life history studies of the Phaeophyta. *Phycologia* 15: 435-452.